

I. AMENDMENT

In the Specification:

Please replace the paragraph beginning on page 18, line 21, with the following amended paragraphs:

Further still, U.S. Pat. No. 5,194,392 to Geysen (1990) describes a general method of detecting or determining the sequence of monomers (amino acids or other compounds) which is a topological equivalent of the epitope (*i.e.*, a "mimotope") which is complementary to a particular paratope (antigen binding site) of an antibody of interest. More generally, U.S. Pat. No. 4,433,092 to Geysen (1989) describes a method of detecting or determining a sequence of monomers which is a topographical equivalent of a ligand which is complementary to the ligand binding site of a particular receptor of interest.

Similarly, U.S. Pat. No. 5,480,971 to Houghten, *et al.* (1996) on Peralkylated Oligopeptide Mixtures discloses linear C₁-C₇ -alkyl peralkylated oligopeptides and sets and libraries of such peptides, as well as methods for using such oligopeptide sets and libraries for determining the sequence of a peralkylated oligopeptide that preferentially binds to an acceptor molecule of interest. Thus, non-peptide analogs of the epitope-bearing peptides of the invention also can be made routinely by these methods.

Please replace Table 2 (beginning on page 21, line 8) with the following amended Table 2:

Table 2: Exemplary Amino Acid Substitutions for Linear Lengthening or Shortening of Side Chains with Un-Natural Amino Acids

Analog of Ala7: (Ala7: R chain = CH₃)

Compound

Reagent Used with side chain amino
acid longer than Natural Glycine (CH₂

= 0)

R chain = CH₂CH₂CH₃

-aminobutyric acid (+1 CH₂)

Fmoc-Abu-OH

N- α -fmoc-L- α -aminobutyric acid

Fmac-2-aminobutanoic acid
C₁₉H₁₉NO₄; M.W.: 325.4

R chain = CH₂CH₂CH₂

Norvaline (+2 CH₂)

Fmoc-Nle-OH

N- α -fmac-L-norvaline

C₂₀H₂₁NO₄; M.W.: 339.4

R chain = CH₂CH₂CH₂CH₃

Norleucine (+3 CH₂)

Fmoc-Nle-OH
N- α -fmac-L-norleucine
CAS No. 77284 32-3; C₂₁H₂₃NO₄;
M.W.: 353.4

**Analogs of Phe8 (Phe8: R chain =
CH₂(C₆H₅))**

Compound

Reagent Used

R chain = C₆H₅

Phenyl Glycine (-1CH₂)

Fmac-Phg-OH
N- α -fmac-L-phenylglycine
C₂₃H₁₉NO₄; M.W.: 373.4

**Analogs of Lys1 (R chain =
CH₂CH₂CH₂CH₂NH₂)**

Compound

Reagent

R chain = $\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$

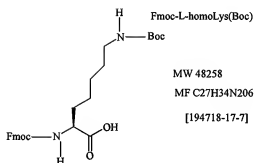
Ornithine ($-\text{CH}_2$)

Fmac-Orn(Bac)-OH

N- α -Fmac-N- δ -Bac-L-ornithine

CAS No: 109425-55-0; $\text{C}_{25}\text{H}_{30}\text{N}_2\text{O}_6$;

M.W.: 454.5



MW 48258

MF C27H34N2O6

[194718-17-7]

R chain = $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$

Homolysine (+1 CH_2)

R chain = $\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{NH}_2$

Analogs of Ile2: (R chain = $\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$)*

Compound

Reagent

R chain: $\text{CH}_2\text{CH}(\text{CH}_3)\text{CH}_2\text{CH}_3$

γ -Methyl L-leucine(+1 CH_2)

H-Leu(γ Me)-OH

γ -Methyl-L-leucine

$\text{C}_7\text{H}_{15}\text{NO}_2$; M.W.: 145.2

Analogs of Phe3: (R chain = $\text{CH}_2(\text{C}_6\text{H}_5)$)

Compound

Reagent Used

R chain = C_6H_5

Phenyl Glycine ($-\text{ICH}_2$)

Fmac-Phg-OH

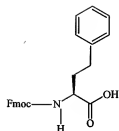
N- α -fmac-L-phenylglycine

$C_{23}H_{19}NO_4$; M.W.: 373.4

R chain: $CH_2CH_2(C_6H_5)$

Homophenylalaine (+1 CH_2)

Fmoc-L-homoPhe



MW 401.47

MF $C_{25}H_{23}NO_4$

[204384-69-0]

Gly4: (R chain = H)**

Analog of Ser5: (R chain = CH_2OH)

CH_2 -Analog of Ser 5 (R chain =

CH_2OH)

Compound

Reagent

R chain: OH

2-amino 2-hydroxy Acetic Acid (-1

CH_2) (unstable under peptide

synthesis conditions)

R chain: CH_2CH_2OH

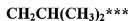
Homoserine (+1 CH_2)

Fmoc-Hse(Trt)-OH

N- α -fmoc-O-trityl-L-homoserine

$C_{38}H_{33}NO_5$; M.W.: 583.7

Analog of Leu6 (R Chain =



<u>Compound</u>	<u>Reagent</u>
R chain: $\text{CH}_2\text{CH}_2\text{CH}(\text{CH}_3)_2$	<u>Homoleucine (-1-CH₂)</u>
	<u>Homoleucine (+1-CH₂)</u>
Ala7: R Chain – CH ₃	Previously tested
See Phe3	
See Leu6	

* Since the first carbon of the R chain is branched, eliminating this carbon to form a (-1 CH) structure would radically affect the makeup of this amino acid and may cause unwarranted side reactions. ** Any alterations in the side chain of this amino acid results in a non-homologous amino acid. *** Removing the first methylene group to make a (-1 CH₂) compound results in the formation of the natural amino acid Valine.

Please replace the paragraph beginning on page 27, line 19, with the following amended paragraph:

For instance, combining synthetic preparation of the immunologically important epitope in the coat protein of foot-and-mouth disease virus combined with assays for immunologic activity, Geysen *et al.* identified the epitope with a resolution of seven amino acids by synthesis of an overlapping set of all 208 possible hexapeptides covering the entire 213 amino acid sequence of the protein. Then, a complete replacement set of peptides in which all 20 amino acids were substituted in turn at every position within the epitope were synthesized, and the particular amino acids conferring specificity for the reaction with antibody were determined. Thus, peptide analogs of the epitope-bearing peptides of the invention can be made by this

method. U.S. Pat. No. ~~4,708,781~~ 4,708,871 to Geysen (1987) further describes this method of identifying a peptide bearing an immunogenic epitope of a desired protein.

Please replace the paragraph beginning on page 39, line 10, with the following amended paragraph:

The nucleotide and protein, polypeptide and peptide encoding sequences for various antigens have been previously disclosed, and may be found at computerized databases known to those of ordinary skill in the art. One such database is the National Center for Biotechnology Information's Genbank and GenPept databases (~~<http://www.ncbi.nlm.nih.gov/>~~ (found on the world wide web at [ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov/))). The coding regions for these known antigens may be amplified and/or expressed using the techniques disclosed herein or by any technique that would be known to those of ordinary skill in the art (*e.g.*, Sambrook *et al.*, 2001). Though a nucleic acid may be expressed in an *in vitro* expression system, in certain embodiments of the invention the nucleic acid comprises a vector for *in vivo* replication and/or expression.

Please replace the paragraph beginning on page 41, line 19, with the following amended paragraph:

Given that many DNA and proteins are known (see for example, the National Center for Biotechnology Information's Genbank and GenPept databases (~~<http://www.ncbi.nlm.nih.gov/>~~ (found on the world wide web at [ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov/))), or may be identified and amplified using the methods described herein, any purification method for recombinantly expressed nucleic acid or proteinaceous sequences known to those of skill in the art can now be employed. In certain aspects, a nucleic acid may be purified on polyacrylamide gels, and/or cesium chloride centrifugation gradients, or by any other means known to one of ordinary skill in the art (see for example, Sambrook *et al.* 2001 incorporated herein by reference). In further aspects, a purification of a proteinaceous sequence may be conducted by recombinantly expressing the

sequence as a fusion protein. Such purification methods are routine in the art. This is exemplified by the generation of an specific protein-glutathione S-transferase fusion protein, expression in *E. coli*, and isolation to homogeneity using affinity chromatography on glutathione-agarose or the generation of a polyhistidine tag on the N- or C-terminus of the protein, and subsequent purification using Ni-affinity chromatography.

Please replace the paragraph beginning on page 65, line 3 with the following amended paragraph:

^aThe binding stability is an estimate of half time of dissociation (in minutes) from HLA-A2 of peptides of the sequence listed above. The theoretical half-life of dissociation was calculated using Parker's algorithm (Parker *et al.*, 1994) available on the world wide web at <http://bimas.dcrt.ig.gov/molbiol/hla-bind> bimas.dcrt.ig.gov/molbiol/hla-bind.

Please amend page 72, line 11 of the specification as follows:

U.S. Patent ~~4,433,092~~4,833,092

Please amend page 72, line 23 of the specification as follows:

U.S. Patent ~~4,708,781~~4,708,871